

Review

Oncolytic herpes simplex virus vectors for cancer virotherapy

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Oncolytic herpes simplex virus type 1 (HSV-1) vectors are emerging as an effective and powerful therapeutic approach for cancer. Replication-competent HSV-1 vectors with mutations in genes that affect viral replication, neuropathogenicity, and immune evasiveness have been developed and tested for their safety and efficacy in a variety of mouse models. Evidence to-date following administration into the brain attests to their safety, an important observation in light of the neuropathogenicity of the virus. Phase I clinical trials of three vectors, G207, 1716, and NV1020, are either ongoing or completed, with no adverse events attributed to the virus. These and other HSV-1 vectors are effective against a myriad of solid tumors in mice, including glioma, melanoma, breast, prostate, colon, ovarian, and pancreatic cancer. Enhancement of activity was observed when HSV-1 vectors were used in combination with traditional therapies such as radiotherapy and chemotherapy, providing an attractive strategy to pursue in the clinic. Oncolytic HSV-1 vectors expressing “suicide” genes (thymidine kinase, cytosine deaminase, rat cytochrome P450) or immunostimulatory genes (IL-12, GM-CSF, etc.) have been constructed to maximize tumor destruction through multimodal therapeutic mechanisms. Further advances in virus delivery and tumor specificity should improve the likelihood for successful translation to the clinic.

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Replication-competent oncolytic herpes simplex virus (HSV) vectors

Over the past decade, since the very first replication-competent oncolytic herpes virus was described in 1991 for the treatment of malignant gliomas,¹ substantial progress has been made in exploiting the unique features of HSV type 1 as vectors for cancer therapy. This progress is amply demonstrated by the translation of three different HSV vectors to the clinic.^{2–4} The pertinent features that allowed HSV-1 to be used as vectors for cancer therapy are still key to the continued development of newer and more efficient vectors. HSV-1 is attractive for cancer therapy because of the following characteristics: (a) it infects a broad range of cell types and species; (b) it is cytolytic by nature (i.e., the replicative life cycle of the virus results in host cell destruction); (c) the well-characterized large genome (152 kb) contains many non-essential genes that can be replaced (up to 30 kb) with multiple therapeutic transgenes;⁵ (d) a number of non-essential genes are associated with neurovirulence;^{6,7} (e) many

antiherpetic drugs are available as a safeguard against unfavorable replication of the virus;⁸ and (f) the virus remains as an episome within the infected cell, even during latency, precluding insertional mutagenesis.⁹ These are distinct advantages over other viral vectors, such as adenovirus, retrovirus, and vaccinia virus.¹⁰

First-generation, single-mutant HSV vectors

One source of replication-competent HSV-1 vector selectivity for actively dividing cells, and hence cancer cells, is by virtue of mutations in viral enzymes involved in nucleotide metabolism (thymidine kinase [TK], ribonucleotide reductase [RR], and uracil deglycosylase [UNG]).^{11–13} There are functional similarities between the viral and cellular enzymes, which are up-regulated in cancer cells and not expressed for the most part in postmitotic cells.^{14–18} Early attempts to utilize HSV-1 as an oncolytic vector focused on mutating/deleting one of these genes, leading to the development of *first-generation* attenuated vectors (Table 1, Fig 1). The first HSV-1 vector genetically engineered for oncolytic therapy, *dlsptk*, contained a deletion of the *TK* gene and was targeted for brain tumor therapy.¹ Athymic mice harboring intracerebral human malignant gliomas when treated with *dlsptk* survived longer, with complete cures seen in some treated animals.¹ However, neurotoxicity at high viral titers and the insensitivity of this mutant to

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Table 1 Oncolytic HSV vectors used for cancer therapy

Virus	HSV genes mutated	Transgenes	Features	References
<i>First-generation vectors</i>				
<i>dlsp tk</i>	<i>TK</i>		Tumor cell complementation	[1,116]
<i>dl8.36tk</i>	<i>TK</i>	<i>LacZ</i>	Tumor cell complementation	[11]
			Track viral replication	
<i>hrR3</i>	<i>ICP6</i>	<i>LacZ</i>	Tumor cell complementation	[19,24]
			Track viral replication	
<i>R3616</i>	γ 34.5		Attenuated for neurovirulence	[27,117,118]
			Reduced viral replication	
<i>1716</i>	γ 34.5		Attenuated for neurovirulence	[31,119]
			Reduced viral replication	
<i>NV1020 (R7020)</i>	<i>UL24, UL56</i>		HSV-1/HSV-2 intertypic recombinant	[34,35]
			Vaccine strain	
<i>Second-generation vectors</i>				
<i>G207 (MGH-1)</i>	<i>ICP6, γ34.5</i>	<i>LacZ</i>	Tumor cell complementation	[38]
			Attenuated for neurovirulence	
			Reduced viral replication	
			Track viral replication	
<i>3616UB</i>	<i>UNG, γ34.5</i>	<i>LacZ</i>	Attenuated for neurovirulence	[120]
			Tumor cell complementation	
			Reduced viral replication	
			Track viral replication	
<i>SUP</i>	γ 34.5, <i>ICP47</i>		Attenuated for neurovirulence	[78]
			No down-regulation of MHC class I	
			Enhanced replication	
<i>NV1023</i>	<i>UL56, ICP47</i>	<i>LacZ</i>	No down-regulation of MHC class I	[74]
			Track viral replication	
<i>Third-generation vectors</i>				
<i>G47Δ</i>	<i>ICP6, γ34.5, ICP47</i>	<i>LacZ</i>	Attenuated for neurovirulence	[33]
			No down-regulation of MHC class I	
			Enhanced replication	
			Tumor cell complementation	
			Track viral replication	
<i>Transcriptionally targeted vectors</i>				
<i>G92A</i>	<i>TK, US3, UL24</i>	<i>LacZ</i> <i>Alb-ICP4</i>	Selectivity for hepatocellular carcinoma	[79]
			Track viral replication	
<i>d12.CALP</i>	<i>TK, US3, UL24</i>	<i>LacZ</i> <i>Cal-ICP4</i>	Insensitive to ACV, GCV	[83]
			Selectivity for soft tissue and bone tumors	
			Track viral replication	
<i>Myb34.5</i>	<i>ICP6</i>	<i>Myb-γ34.5</i>	Insensitive to ACV, GCV	[80]
			Selectivity for cycling tumor cells	
			Tumor cell complementation	
<i>Transgene-expressing vectors</i>				
<i>rRp450</i>	<i>ICP6</i>	<i>CYP2B1</i>	Tumor cell complementation	[67]
			Bystander effect of toxic metabolite CPA	
<i>HSV1yCD</i>	<i>ICP6</i>	<i>CD, AFP</i>	Tumor cell complementation	[68]
			Bystander effect of toxic metabolite 5-FU	
<i>NV1042</i>	<i>UL56, ICP47</i>	<i>IL-12</i> <i>LacZ</i>	mIL-12 expression from α_4 promoter	[74]
			No down-regulation of MHC class I	
			Track viral replication	
<i>NV1034</i>	<i>UL56, ICP47</i>	<i>GM-CSF</i> <i>LacZ</i>	mGM-CSF expression from α_4 promoter	[74]
			No down-regulation of MHC class I	
			Track viral replication	
<i>R8306</i>	γ 34.5	<i>IL-4</i>	mIL-4 expression from <i>egr-1</i> promoter	[121]
<i>R8308</i>	γ 34.5	<i>IL-10</i>	mIL-10 expression from <i>egr-1</i> promoter	[121]
<i>M002</i>	γ 34.5	<i>IL-12</i>	mIL-12 expression from <i>egr-1</i> promoter	[76]
<i>JS1/- (OncoVEX [GM-CSF])</i>	γ 34.5, <i>ICP47</i>	<i>GM-CSF</i>	mGM-CSF expression from CMV promoter	[122]
			No down-regulation of MHC class I	

ACV, acyclovir; AFP, autofluorescence protein; Alb, albumin promoter/enhancer; Cal, calponin promoter; CD, yeast cytosine deaminase; CPA, cyclophosphamide; *CYP2B1*, rat cytochrome P450; 5-FU, 5-fluorouracil; GCV, ganciclovir; *LacZ*, *E. coli LacZ*; myb, B-*myb* promoter.



Figure 1 HSV DNA structure and schematic arrangement of genes mutated in the different oncolytic HSV vectors. The boxes represent the inverted repeat sequences (TR and IR) flanking the long (L) and short (S) unique regions. Arrows indicate the orientation and general position of the indicated transcripts.

antiherpes drugs, such as acyclovir (ACV) and ganciclovir (GCV), due to the absence of TK were major drawbacks to the clinical use of this vector. Nevertheless, the *dlsptk* mutant served as a proof-of-principle that attenuated mutants of HSV-1 have therapeutic potential and formed the launching pad for further studies. The safety feature afforded by the presence of the TK gene in HSV-1 mutants was important to the clinical application of these vectors to treat human cancers. Hence, the focus for further development of HSV-1 vectors was directed at engineering viruses with mutations in other genes affecting virulence.

A RR-negative mutant, hrR3, with an in-frame insertion of the *Escherichia coli LacZ* gene into the *ICP6* locus (Fig 1), was one such vector.¹⁹ The presence of *LacZ* provides an efficient mechanism to track viral infection and to distinguish the vector from patient isolates. *ICP6*⁻ mutants exhibit decreased neurovirulence^{20,21} and are hypersensitive to ACV and GCV.^{22,23} In experimental animal models, hrR3 was effective in treating malignant gliomas, prolonging survival times.^{24,25} Another set of HSV-1 vectors contains deletions in the $\gamma 34.5$ gene (Fig 1), the major viral determinant of neuropathogenicity.^{26,27} The $\gamma 34.5$ gene product blocks the shut-off of host cell protein synthesis induced by viral infection.²⁸ Expression of this protein also facilitates viral replication in nondividing cells, such as adult neurons, contributing to the development of encephalitis.^{29,30} HSV-1 $\gamma 34.5$ deletion mutants include 1716, in strain 17⁺,³¹ and R3616, in strain F,²⁷ both of which have been used successfully to treat a variety of animal tumor models.⁷ One potential drawback of $\gamma 34.5$ mutants is that they replicate less efficiently, with lower viral yields, as compared to wild-type virus.^{32,33}

Another attenuated HSV-1 mutant currently being studied extensively for cancer therapy is NV1020 (previously known as R7020), in which the joint region of the long (L) and short (S) regions is deleted, including one copy of $\gamma 34.5$, *UL24*, and *UL56*.^{34,35} The deleted region was replaced with a fragment of HSV-2 US DNA (*US2*, *US3* (PK), *gJ*, and *gG*). This virus was originally developed as a herpes vaccine but was unsuccessful. However, building on the associated safety studies in rodents and primates,^{34,36} it has been used as an oncolytic agent against various non-CNS tumors (prostate, pancreatic, head and neck).^{35,37} These first-generation HSV-1 vectors, thus, provided the foundation for examining the critical issues of safety, specificity, and efficacy for oncolytic virotherapy.

Second-generation, multimutated HSV vectors

In order to maximize safety, it was reasoned that HSV-1 vectors developed for clinical application contain multiple

mutations, so that virulent strains would not arise from reversion or second site suppressor mutations (Table 1). G207 was constructed as a second-generation vector from HSV-1 laboratory strain F, with both copies of $\gamma 34.5$ deleted and the *ICP6* gene inactivated by insertion of the *E. coli LacZ* gene.³⁸ Experimental testing of G207 in animal models for the treatment of malignant gliomas attested to its efficacy without compromising safety or specificity.³⁸ Treatment of athymic mice, harboring established intracerebral or subcutaneous human U87MG gliomas, with a single intraneoplastic inoculation of G207 resulted in significant tumor regression prolonging their survival time. Following inoculation into tumors, G207 replicated preferentially in glioma cells but not in normal tissues, as monitored by histochemical detection of β -galactosidase (*LacZ*).³⁹ Although G207 was initially targeted for brain tumor therapy, experimental studies *in vitro* and *in vivo* showed that it was equally, if not more, effective against a wide variety of solid tumors, including melanoma, breast, colon, gallbladder, gastric, head and neck, ovarian, pancreatic, and prostate cancers.⁴⁰ In syngeneic orthotopic bladder cancer and colon cancer metastatic to the liver models, G207 and NV1020 were equally effective.^{41,42} Interestingly, in immune-competent mice, intraneoplastic inoculation of G207 into a local syngeneic tumor was found to induce systemic antitumor immunity, leading to the regression of a distant tumor and resistance to rechallenge with autologous tumor cells.⁴³ Thus, G207 acts as an *in situ* cancer vaccine in these systems.

As data continued to accumulate regarding the antitumor efficacy of G207, preclinical toxicology studies were also conducted. Two animal models known for their exquisite sensitivity to HSV were chosen for safety testing: young BALB/c mice and *Aotus nancymai*, New World owl monkeys. As the natural tropism for HSV-1 is the central nervous system, extensive toxicity evaluation was conducted after administration into the brain. In mice, doses of up to 1×10^7 plaque-forming units (pfu) of G207 inoculated into the brain (cerebrum or the ventricles), liver, or prostate or through intravenous delivery resulted in no adverse effects.^{44,45} G207 was also inoculated into peripheral nerves without leading to any nerve damage, thereby demonstrating that it is a nerve-sparing virus.⁴⁶ In *Aotus* monkeys, a dose as high as 1×10^9 pfu of G207 inoculated intracerebrally did not result in significant disease or pathology, whereas a dose of 1×10^3 pfu of parental strain F caused encephalitis and serious morbidity within 5 days.⁴⁷ Two of these G207-inoculated animals were injected intraprostatically with G207 2 years later.⁴⁵ In this study, 1×10^7 pfu of G207 was inoculated, either in the previously exposed or naïve monkeys, and did not result in any abnormalities or virus shedding. The analysis included histopathology of the brain, liver, lung,

kidney, urogenital organs, *etc.*; PCR analysis of the various tissues for the presence of viral DNA; viral shedding; and blood analysis (differential and chemistry).

Combination with conventional therapies

In so far as the selection criterion for engineering replication-competent attenuated HSV-1 vectors is to target actively dividing cells, it is similar to conventional modes of cancer therapy such as chemotherapy and radiotherapy. However, with chemotherapy and radiotherapy, the narrow range of the therapeutic index, coupled with limiting high-dose toxicities, severely restricts their effectiveness. In contrast, viruses such as G207 have been efficacious in destroying tumors without toxicity to the host.⁴⁰ Moreover, because tumors are typically heterogenous genetically and therapy selects for resistant phenotypes, no single agent is usually universally applicable or completely effective. The mode of action of HSV-1 vectors, however, is different from that of standard therapies and is independent of many of the genotypic alterations, such as in p53, which are observed with chemotherapy or radiation-resistant tumors.^{48,49} Additionally, the maximal effect of replicating viruses should increase with time, whereas the peak activity of drugs declines with time. G207, NV1020, and 1716 have been tested in combination with standard chemotherapeutic agents^{50,51} or with ionizing radiation^{52–55} for augmentation of their individual efficacies.

The combined regimen of G207 and cisplatin was more effective (100% cure) than either cisplatin (14% cure) or G207 alone (42% cure) in treating cisplatin-sensitive human head and neck squamous cell carcinoma in an animal model.⁵⁰ An additive efficacy was observed with 1716 and mitomycin C in a human lung cancer model.⁵¹ Ionizing radiation enhanced the lytic activity of NV1020 (R7020) in two separate models: human glioma⁵³ and hepatoma⁵⁴ xenografts in athymic mice. However, the effect in hepatoma was tumor cell-specific, in that the combined therapy had an effect on Hep3B but not on HuH7 tumors.⁵⁴ The response with G207 and radiation is ambiguous; in one set of studies with human or syngeneic mouse prostate tumors, there was no enhanced efficacy with combination treatment,⁵⁹ whereas in a cervical cancer model, increased efficacy was observed.⁵⁵ The augmenting effects seen in the above combination modalities, likely based on the noninterfering activity of each agent, are very promising and have potential for immediate translation to the clinic.

Similar augmentation of efficacy has also been observed with oncolytic adenovirus vectors such as ONYX-015 (*d11520*). The combination with cisplatin or 5-fluorouracil in mouse models was better than either agent alone.⁵⁶ More importantly, clinical trials of ONYX-015 in combination with cisplatin and 5-fluorouracil for head and neck cancer or metastatic colorectal cancer have provided encouraging results for this strategy.^{57,58} Interestingly, therapeutic doses of chemotherapy do not seem to inhibit the replication of any of the oncolytic viruses. The combination of ONYX-015 with radiation produced an additive antitumor effect in a radiation-sensitive, ONYX-015-sensitive, p53 mutant tumor cell line, but had no effect in the matched p53 wild-type, ONYX-015-resistant tumor cell line.⁶⁰

Modified oncolytic HSV vectors for enhanced activity or targeting

Prodrug-activating HSV-1 vectors

Strategies aimed at using prodrug-activating enzyme or “suicide” gene therapy initially focused on using the *HSV-TK* gene to convert GCV into toxic metabolites⁶¹ (Table 1). The presence of TK in HSV-1 vectors imparts an inherent sensitivity to GCV such that both infected cellular and viral replications are blocked. However, this effect can be counterproductive to HSV-1 oncolytic therapy because it inhibits viral spread prematurely. The addition of GCV to hrR3 treatment of 9L tumor-bearing animals prolonged survival.^{25,62} However, in other experimental model systems, the combination of HSV-1 oncolysis and GCV treatment was not superior to HSV-1 oncolysis alone.^{63–66} Other prodrug-activating enzymes that have been utilized with oncolytic HSV-1 are rat cytochrome P450 (*CYP2B1*), which converts cyclophosphamide (CPA) to phosphoramidate mustard (PM),⁶⁷ and cytosine deaminase, which converts 5-fluorocytosine to 5-fluorouracil⁶⁸ (Table 1). Unlike GCV triphosphate, PM causes minimal inhibition of viral replication and can diffuse to neighboring cells.⁶⁷ An HSV-1 mutant, rRp450, carrying the *CYP2B1* transgene inserted into the *ICP6* locus, exhibited enhanced efficacy in combination with CPA in suppressing the growth of subcutaneous glial tumors or diffuse liver tumors in rodents.^{67,69} However, expression of *CYP2B1* in normal liver cells, where conversion of CPA could lead to toxic side effects, is a consideration against systemic use of this virus.

Immunostimulatory HSV-1 vectors

In immunocompetent animals, the therapeutic efficacy of oncolytic HSV-1 vectors appears to encompass two modes of action: direct cytotoxic activity of the virus itself and indirect induction of an antitumor immune response. This bimodal effect was demonstrated using bilateral syngeneic tumor models, where tumor growth inhibition occurred in both the virus-inoculated and noninoculated contralateral tumors.^{43,70} The antitumor immune response included a CD8⁺ T-cell component generated against specific tumor cell antigens,⁴³ and provided protection against rechallenge with a lethal dose of the same tumor cells.⁷⁰ Thus, even as improvements are being made to enhance the lytic efficacy of the virus, efforts are also directed at promoting this antitumor immune response. Accordingly, many recombinant HSV vectors have been developed with the goal of exploiting the immune milieu around the tumor through genetically engineering HSV-1 either to abrogate viral-induced MHC class I down-regulation (by deletion of *ICP47*) or to deliver regulatory cytokines. Alternatively, oncolytic HSV vectors have been used as helper viruses for the generation of defective HSV vectors expressing immunomodulatory genes, such as *IL-2*,⁷¹ *IL-12*,⁷² or soluble *B7.1-Ig*.⁷³ This latter strategy has certain advantages because the defective vector genome contains multiple copies of the transgene and cells infected by the defective vector alone will not be immediately killed as occurs after infection with the oncolytic vector. Among the recombinant vectors expressing cytokines, NV1034 and NV1042 are

derivatives of NV1020, containing two additional insertions: the *E. coli* *LacZ* gene within *ICP47* and either GM-CSF (NV1034) or mIL-12 (NV1042)^{74,75} (Table 1). Both GM-CSF and IL-12 were produced, respectively, by NV1034- or NV1042-infected murine squamous cell carcinomas *in vivo*; however, NV1042 was much more effective at inhibiting tumor growth.⁷⁴ Another IL-12-expressing HSV-1 vector, M002, derived from the $\gamma 34.5$ deletion mutant R3659, exhibited increased efficacy against murine brain tumors, with a significant infiltration of CD4⁺ and CD8⁺ T cells and macrophages.⁷⁶

G47 Δ is a *third-generation* vector that was constructed from G207 by deletion of the *ICP47* gene (Fig 1), which normally blocks MHC class I-mediated antigen presentation in infected cells³³ (Table 1). Consequently, human melanoma cells infected with G47 Δ expressed higher levels of MHC class I on their surface, compared to G207-infected cells, resulting in enhanced stimulation of tumor-infiltrating lymphocytes. The *ICP47* deletion also removes the *US11* promoter, so that the late *US11* gene is expressed as an immediate-early gene under the control of the *ICP47* promoter, thereby suppressing the diminished growth properties of $\gamma 34.5$ mutants.⁷⁷ This improved replication of G47 Δ translates into enhanced antitumor activity.^{33,78}

Transcriptionally targeted vectors

Targeting the replication of oncolytic viruses to the tumor is essential for maximal benefit, especially when delivered systemically. To date, two strategies have been employed to target viral replication: the use of a tumor tissue-specific promoter/enhancer to drive expression of the essential immediate-early gene *ICP4*⁷⁹ (replication-conditional vectors), or to express $\gamma 34.5$ ⁸⁰ to enhance replication in a fashion complementary to the *ICP47* deletion (Table 1). Myb34.5, a transcriptionally potentiated vector, is derived from the $\gamma 34.5$ deletion mutant, MGH-1, by insertion of an exogenous $\gamma 34.5$ transgene driven by the E2F-responsive B-*myb* promoter into the *ICP6* locus.⁸⁰ Therefore, it would be expected to target cycling cancer cells and also replicate more efficiently than the $\gamma 34.5$ -deleted parental virus.

Myb34.5 was more efficacious than MGH-1 after intratumoral injection of subcutaneous gliomas or intravascular delivery to liver metastases.^{80,81} However, safety was partially compromised compared to $\gamma 34.5$ mutants.

G92A was the first example of a transcriptionally targeted HSV vector, where the albumin enhancer/promoter driving *ICP4* was used to specifically target hepatocellular carcinomas.⁷⁹ The specificity of G92A was demonstrated *in vitro* and *in vivo* where it selectively inhibited the growth of subcutaneous human Hep3B hepatoma tumors but not human PC3 prostate tumors.⁸² Importantly, intrahepatic delivery of G92A (*TK*⁻ and *US3*⁻) did not result in toxicity in the liver, as opposed to wild-type KOS virus, indicating the sparing of normal tissue by G92A virus.⁸² More recently, another *ICP4*-driven vector, *d12.CALP*, has been generated using the calponin promoter to drive HSV replication in soft tissue and bone tumors.⁸³ A similar transcriptionally regulated replication strategy has been described for adenovirus vectors, termed CRAAd (conditionally replicative adenovirus) or ARCA (attenuated, replication-competent adenovirus). Specificity has been achieved using multiple tissue- or tumor-specific promoters (i.e., α -fetoprotein, kallikrein, L-plastin, midkine, prostate-specific antigen, tyrosinase) to regulate the expression of early genes *E1A* or *E1B*.^{84,85} These CRAAd vectors have also been used in combination with standard therapies such as chemotherapy and radiation. In a limited number of studies so far, the combinations have synergistically enhanced oncolytic activity.⁸⁶⁻⁸⁸

Clinical trials

Successful preclinical efficacy and safety studies have facilitated the launching of three oncolytic HSV-1 vectors into clinical trials: G207, 1716, and NV1020 (Table 2). Phase I clinical trials with G207, conducted in the US (Georgetown University and University of Alabama at Birmingham), and 1716, conducted in the UK (University of Glasgow), have been completed and were published in parallel in 2000.^{2,3} In both cases, patients with recurrent malignant glioma, refractory to conventional treatments of

Table 2 Oncolytic HSV vectors in clinical trial

Virus	Disease (patient number)	Delivery	Maximum dose (pfu)	Comments	References
G207	Recurrent glioma (21)	Intratumoral	3 $\times 10^9$	Safety confirmed MRI volume decrease in eight patients Two patients alive over 4 years Ongoing trial	[2]
1716	Recurrent glioma	Intratumoral, resection bed	1 $\times 10^5$	Safety confirmed	[3]
	Recurrent glioma (9)	Intratumoral		Four patients alive over 14 months	
	HGG (12)	Intratumoral		Safety confirmed	[89]
	Melanoma (5)	Intralesional		Evidence for viral replication IHC evidence of viral replication Some tumor necrosis	[90]
NV1020	Hepatic metastatic colorectal cancer (9)	Hepatic artery	1.3 $\times 10^7$	Decrease in CEA MTD not reached Ongoing trial	[4]

CEA, carcinoembryonic antigen; HGG, high-grade glioma; IHC, immunohistochemistry; MRI, magnetic resonance imaging; MTD, maximum tolerated dose.

radiation and chemotherapy, were treated. A dose-escalating phase I clinical trial with NV1020 is ongoing for colorectal liver metastases, with the virus administered through the hepatic artery.⁴ Recently, BioVex has obtained approval to initiate a phase I clinical trial of OncoVEX(GM-CSF), a $\gamma 34.5$ deletion vector expressing GM-CSF (Table 1), for the treatment of solid tumors. Currently, clinical trials are conducted on patients with advanced forms of disease, so that long-term safety assessments are not completely available. Nonetheless, the results so far from all the clinical trials have been very promising, with no evidence of serious toxicity attributable to the virus while demonstrating anecdotal evidence of efficacy.

G207 clinical trials

The G207 phase I trial included 21 patients who received doses ranging from 1×10^6 to 3×10^8 pfu in 0.1 mL and 1×10^9 pfu in 0.3 mL at a single site with a final cohort receiving 3×10^9 pfu inoculated at five sites (0.2 mL each).² Three patients were enrolled per dose and the virus was administered stereotactically to a contrast-enhancing region of the tumor (Fig 2). All of the patients tolerated G207 without any serious adverse events attributable to the virus. No viral shedding was detected, but G207 DNA was detected by PCR analysis in two of seven patients who underwent brain tumor biopsy (56 and 157 days posttreatment). The presence of viral DNA at these later times after virus inoculation is likely due to "latently" or nonproductively infected cells. Of the five patients who were HSV-1-seronegative prior to G207 inoculation, one patient seroconverted.² Interestingly, eight patients exhibited radiographic reduction in tumor volume from 4 days to 1 month postinoculation. Two patients are still alive over 4 years after treatment and one patient who died from a cerebral

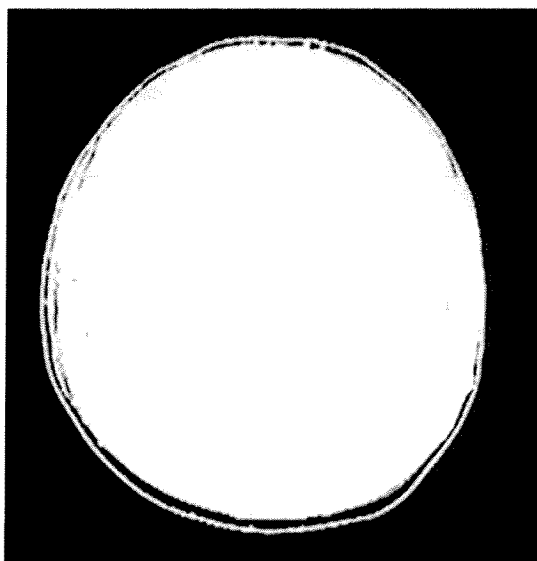


Figure 2 Axial contrast-enhanced computed tomography (CT) scan of patient's glioma prior to G207 injection. The target site for stereotactic injection is within an enhancing region of the tumor, as indicated (◊). Photo courtesy of T Mineta.

infarction (unrelated to treatment) at 10 months posttreatment did not show any evidence of residual tumor mass at autopsy.² None of the patients who died displayed any signs of encephalitis during postmortem analysis. G207 is currently in a phase Ib trial where G207 is inoculated into the tumor and then, 2 days later, the tumor is resected and the tumor bed is inoculated. The resected tumor will then be analyzed for evidence of viral replication. A planned phase II trial will involve delivery to the tumor bed after tumor resection in order to improve distribution of the virus and decrease the tumor mass that needs to be destroyed. As the current standard of care for malignant glioma patients is ineffective, clinical trials in patients with newly diagnosed glioma, as opposed to recurrent, previously treated disease are a possibility. These patients are more likely to experience positive clinical outcomes from HSV therapy.

1716 clinical trials

The 1716 trial tested a lower dose range (1×10^3 – 1×10^5 pfu) in nine patients with recurrent gliomas³ (Table 2). Patient selection criteria were similar to the G207 trial; however, virus delivery was different with multiple injections of a 1-mL total volume. No virus was detected by PCR in five patients and none exhibited any adverse events. Promisingly, four patients were alive over 14 months after treatment. Recently, a second trial of 1716 for malignant glioma was reported.⁸⁹ Twelve patients with high-grade glioma (HGG) were enrolled, of which all, except for one, had recurrent tumors. Patients were injected intratumorally with 1×10^5 pfu 1716, following which the tumor was surgically resected after 4–9 days. Viral replication was demonstrated by recovery of infectious virus (two patients) at levels higher than were inoculated, detection of significant amounts of viral DNA by PCR (10 patients), and immunohistochemical staining for HSV antigens (two patients), including against *UL42*, an early gene product not present in virions.⁸⁹ Interestingly, both seronegative patients seroconverted after treatment and their tumors stained positive for HSV antigens. Unfortunately, they died due to tumor progression within 9 months of treatment. As in the first trial, no adverse events were noted in any of the patients following 1716 injection.⁸⁹ 1716 has also been clinically tested in five patients with stage 4 melanoma, with each patient receiving between one and four injections of 1×10^3 pfu into a single nodule.⁹⁰ In the three patients receiving multiple injections, there was histopathological evidence of tumor necrosis and HSV antigen was detected immunohistochemically only in tumor cells.

Key hurdles

Drawing an analogy to putting a man on the moon, French Anderson noted, "NASA can draw pictures of a spacecraft, with rocket engines and a capsule containing astronauts, going from earth to the moon. But in fact, there are hundreds of critical steps. . . Just having sufficiently powerful rocket engines is not enough. . ."⁹¹ The encouraging results from the clinical trials with oncolytic HSV vectors offer concrete proof that at least some of the past limitations have become

today's possibilities and that the introduction of replication-competent HSV into humans can be performed safely. Nonetheless, major hurdles will have to be conquered before this form of therapy attains the status of conventional therapy. The limitations in using oncolytic HSV vectors for cancer therapy include some that are unique to HSV and some that apply to all replicating vectors; the discussion herein will emphasize the former.

Virus delivery

Most initial studies testing efficacy of HSV-1 vectors were conducted by administering the virus intratumorally. Easily accessible solid tumors are amenable to intratumoral delivery, but this approach is of limited value for those tumors that are not easily accessible, or for metastatic tumors. Multiple routes of oncolytic HSV-1 administration have been tested and found to be effective in animal models, including intravenous,⁹² intra-arterial,⁹³ lymphatic,⁹⁴ intraperitoneal,⁹⁵ and local vascular perfusion.⁹⁶ Whereas additional safety studies will be needed to evaluate these different routes of delivery, so far, the efficacy and lack of toxicity attest to the potential for systemic delivery of oncolytic HSV vectors for virotherapy. Nevertheless, further improvements are required to optimize effective tumor targeting. Rapid inactivation of the virus due to instability (virus half-lives), adsorption, and homing to nonspecific cells; clearing by the liver; innate immunity; preexisting immunity (antibodies) leading to complement-mediated inactivation; and lack of tropism are issues that merit continued research. Increasing the input dose is one way to overcome vector losses during delivery; however, with current manufacturing processes, attainable titers for oncolytic HSV vectors are $\sim 3 \times 10^9$ pfu/mL. Therefore, it is unlikely that patients will receive doses in excess of 10^{10} pfu, as opposed to conditionally replicating adenovirus, which has been used at doses of 10^{11} pfu or higher.^{58,97,98} Assault by host factors on the virus could be minimized by developing "Trojan horse" vectors, which could include the use of novel technologies, such as the use of cell carriers⁹⁹ or chemical "coating" to deliver viruses. Alternatively, the host can be modified by transient suppression of immunity during virus dispensation,⁹³ or by administering virus premixed with complement inhibitory agents (e.g., dextran sulphate¹⁰⁰), etc. Preexisting antibody to HSV is an important consideration as the majority of the human population are seropositive; however, studies in animal models demonstrate that prior immunity does not significantly interfere with therapeutic efficacy.^{42,101–103} On the other hand, general immunosuppression induced by corticosteroid administration was found to reduce the rate of tumor cures.¹⁰⁴ In contrast to HSV, preexisting antibodies to adenovirus have been shown to abrogate the antitumor activity of prostate cancer-specific adenovirus.¹⁰⁵

Tissue/tumor specificity

Efforts are being made to alter the tropism of HSV to specific tumor tissues. Two strategies that need not be mutually exclusive could be envisioned: (a) altering the tropism of the virus by modifying viral receptors to bind specific tumor cell

surface molecules,¹⁰⁶ as has been done with adenovirus vectors;¹⁰⁷ and (b) targeting viral replication within tumor cells using tumor- or tissue-specific promoter elements. Altering viral tropism for a large virus like HSV with multiple essential glycoproteins involved in interactions with various cell surface molecules will be difficult, as illustrated by the initial unsuccessful attempt using erythropoietin.¹⁰⁸ This is in contrast to adenovirus where great advances have been made in developing viral vectors with modifications to the viral coat proteins.¹⁰⁷ The second strategy of targeting replication within tumor cells, as with G92A virus, has already been discussed. As novel tumor-associated genes and their control elements are discovered, HSV-1 vectors can be designed to replicate selectively in these tissues. If high specificity can be achieved, it should be possible to use less attenuated mutants or even wild-type HSV as a backbone to express the tumor-regulated transgene.

Viral replication and spread within the tumor

A major benefit of using replication-competent vectors over defective vectors is the amplification of the input dose within the tumor. The rate of viral replication, however, is dependent on many factors including, the rate of tumor growth and the cellular environment. Targeting tumor cell physiology is a likely way to enhance oncolytic activity.¹⁰⁹ Alterations in the *ras* pathway and interferon signaling have provided tumor selectivity for oncolytic reovirus,¹¹⁰ vesicular stomatitis virus,^{111,112} and influenza A mutants.¹¹³ For viruses deleted/mutated in genes involved in nucleotide metabolism (i.e., RR, TK), one could transiently increase expression of the complementing cellular enzymes,¹⁶ thereby enhancing viral replication. Conversely, a virus strain conditioned to grow in tissue culture in rapidly dividing cells and enriched growth conditions may not replicate as well in a tumor *in vivo*.

Suboptimal spread of the inoculated and newly replicated virus resulting in partial responses is a major challenge with HSV-1 vectors. During the early phases of viral infection, physical barriers play a major role, but with time, both physical and immune barriers restrict the efficient spread of the virus within a solid tumor. Extracellular matrix changes, tight gap junctions, fibrosis, and necrotic regions commonly observed in solid tumors could potentially limit viral spread. The development of syncytial viruses¹¹⁴ and viruses that express enzymes that break down extracellular matrix are options to consider in order to overcome this problem. A second determinant in the efficient spread of virus is antiviral immunity. Innate or acquired, preexisting or induced, immunity would result in immune-mediated clearance of the virus before its effect can be realized. In this regard, viruses that spread predominantly cell-to-cell rather than those that are released would not be affected by neutralizing antibody. When using immunostimulatory vectors, it can be expected that an enhanced immune response will be generated against both the virus and tumor. Ideally, it would be beneficial to generate minimal immune responses against the virus, and hence, methods to skew the response in favor of the tumor are worth considering. It is also important to develop vectors that promote long-term "memory" against

tumors, as demonstrated with G207, where it acts as an *in situ* tumor vaccine.⁷⁰

Combination therapies

As the mechanisms of action of therapeutic viruses are distinct from chemo- or radiotherapeutic agents, additive or synergistic responses are to be expected. Possible concerns about the combination of oncolytic HSV-1 vectors with traditional therapies are that the two might be interfering or that the combination would increase toxicity to normal tissue. For example, the extensive necrosis caused by chemotherapy or radiation could physically limit virus spread or induce an inflammatory response limiting virus spread. The importance of timing in combination therapy was demonstrated with adenovirus vector, ONYX-015, and cisplatin, where maximal efficacy was observed when cisplatin was administered concomitantly with, or subsequent to but not prior to, virus inoculation.¹¹⁵

Viral toxicity/safety

To date, the oncolytic HSV vectors have had an excellent safety profile both preclinically and clinically. Nevertheless, as more virulent vectors are created, similar preclinical evaluations will have to be conducted in appropriate animal models to ensure that the increased efficacy is not at the cost of safety. A distinct advantage of HSV is the availability of sensitive animal models (BALB/c or A/J mice, and the New World owl monkey, *A. nancyai*) that mimic the susceptibility pattern of humans to HSV-1. Safety evaluations should consider reversion to or acquiring virulent phenotypes, novel or previously unrecognized toxicity due to altered tropism or transgene expression, multiple dose-induced inflammatory responses, etc. The immunostimulatory viruses, expressing specific cytokines (IL-12, GM-CSF, etc.), might be expected to alter the host immunoregulatory balance in a haplotype-specific fashion. Hence, toxicological evaluations of these immune-enhanced viruses should be conducted in multiple strains of mice. Moreover, the ectopic overexpression of cytokines in normal tissues might lead to inflammatory responses to self-antigens and, therefore, autoimmunity.

Summary

Oncolytic HSV-1 vectors have proven in a relatively short span of time to be valuable tools for cancer therapy. As the first genetically engineered virus developed for virotherapy,¹ HSV vectors have served as a prototype for the development of other oncolytic viruses. In animal models, oncolytic HSV vectors have proven to be both safe and efficacious through various routes of delivery, including systemic, which is essential for treating advanced metastatic disease. Their efficacy has been enhanced both by direct manipulation of the viral genome and expression of transgenes with cytotoxic or immunomodulatory functions. Synergistic and/or additive effects of oncolytic HSV-1 have also been demonstrated in combination with conventional modes of therapy, such as chemotherapy and radiation. The

phase I clinical trials are beginning to validate the preclinical safety studies while also providing a glimmer of efficacy. Many hurdles remain before this strategy becomes a standard therapeutic reality; however, the studies to date provide ample optimism.

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